DATE-ISSUED: October 30, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cronin; Maureen T.	Los Altos	CA		
Miyada; Charles G	San Jose	CA		
Hubbell; Earl A.	Los Angeles	CA		
Chee; Mark	Palo Alto	CA		
Fodor; Stephen P. A.	Palo Alto	CA		
Huang; Xiaohua C.	Mountain View	CA		
Lipshutz; Robert J.	Palo Alto	CA		
Lobban; Peter E.	Mountain View	CA		
Morris; MacDonald S.	Felton	CA		
Sheldon; Edward L.	San Diego	CA		

US-CL-CURRENT: 435/6; 422/50, 422/68.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 6. Document ID: US 6232065 B1

L7: Entry 6 of 15 File: USPT

US-PAT-NO: 6232065
DOCUMENT-IDENTIFIER: US 6232065 B1

TITLE: Analysis of gene family expression

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Robinson; Daniel R.	Cleveland	OH		
Kung; Hsing-Jien	Chagrin Falls	OH		

US-CL-CURRENT: 435/6; 435/91.2, 536/23.1, 536/24.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 7. Document ID: US 6071743 A

L7: Entry 7 of 15 File: USPT

US-PAT-NO: 6071743
DOCUMENT-IDENTIFIER: US 6071743 A

TITLE: Compositions and methods for inhibiting human immunodeficiency virus infection by down-regulating human cellular genes

DATE-ISSUED: June 6, 2000

☐ 3. Document ID: US 6326141 B1

L7: Entry 3 of 15 File: USPT

US-PAT-NO: 6326141
DOCUMENT-IDENTIFIER: US 6326141 B1

TITLE: Methods for determining risk for Type I and Type II diabetes

DATE-ISSUED: December 4, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kahn; C. Ronald	West Newton	MA		
Reynet; Christine	Boston	MA		

US-CL-CURRENT: 435/6; 435/7.1, 536/23.2, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc
Image

☐ 4. Document ID: US 6312922 B1

L7: Entry 4 of 15 File: USPT

US-PAT-NO: 6312922
DOCUMENT-IDENTIFIER: US 6312922 B1

TITLE: Complementary DNAs

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Edwards; Jean-Baptiste Dumas Milne	Paris			FR
Duclert; Aymeric	Saint Maur			FR
Bougueleret; Lydie	Vanves			FR

US-CL-CURRENT: 435/69.1; 435/252.3, 435/254.11, 435/320.1, 435/325, 435/419, 536/23.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc
Image

☐ 5. Document ID: US 6309823 B1

L7: Entry 5 of 15 File: USPT

US-PAT-NO: 6309823
DOCUMENT-IDENTIFIER: US 6309823 B1

TITLE: Arrays of nucleic acid probes for analyzing biotransformation genes and methods of using the same

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☐ 1. Document ID: US 6344322 B1

L7: Entry 1 of 15 File: USPT

US-PAT-NO: 6344322
DOCUMENT-IDENTIFIER: US 6344322 B1

TITLE: Subtle mitochondrial mutations as tumor markers

DATE-ISSUED: February 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Polyak; Kornelia	Brookline	MA		
Vogelstein; Bert	Baltimore	MD		
Kinzler; Kenneth W.	BelAir	MD		

US-CL-CURRENT: 435/6; 435/366, 435/91.1, 435/91.2, 536/23.1, 536/24.3, 536/24.33

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6326152 B1

L7: Entry 2 of 15 File: USPT

US-PAT-NO: 6326152
DOCUMENT-IDENTIFIER: US 6326152 B1

TITLE: Compositions and methods for inhibiting human immunodeficiency virus infection by down-regulating human cellular genes

DATE-ISSUED: December 4, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Holzmayer; Tanya A.	Mountain View	CA		
Dunn; Stephen J.	Mountain View	CA		
Dayn; Andrew	Mountain View	CA		

US-CL-CURRENT: 435/6; 435/236, 435/26, 435/325, 435/7.1, 435/7.71

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Holzmayer; Tanya A.	Mountain View	CA		
Dunn; Stephen J.	Mountain View	CA		
Dayn; Andrew	Mountain View	CA		

US-CL-CURRENT: 435/325; 435/320.1, 435/366, 536/23.1, 536/24.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 8. Document ID: US 6046044 A

L7: Entry 8 of 15 File: USPT

US-PAT-NO: 6046044
DOCUMENT-IDENTIFIER: US 6046044 A

TITLE: Cisplatin resistance proteins

DATE-ISSUED: April 4, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yokoyama; Shiro	Machida-City, Tokyo 195			JP

US-CL-CURRENT: 435/189; 530/300, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 9. Document ID: US 6027880 A

L7: Entry 9 of 15 File: USPT

US-PAT-NO: 6027880
DOCUMENT-IDENTIFIER: US 6027880 A

TITLE: Arrays of nucleic acid probes and methods of using the same for detecting cystic fibrosis

DATE-ISSUED: February 22, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cronin; Maureen T.	Los Altos	CA		
Miyada; Charles Garrett	San Jose	CA		
Hubbell; Earl A.	Mountain View	CA		
Chee; Mark	Palo Alto	CA		
Fodor; Stephen P. A.	Palo Alto	CA		
Huang; Xiaohua C.	Mountain View	CA		
Lipshutz; Robert J.	Palo Alto	CA		
Lobban; Peter E.	Palo Alto	CA		
Morris; Macdonald S.	Felton	CA		
Sheldon; Edward L.	San Diego	CA		

US-CL-CURRENT: 435/6; 422/50, 422/68.1, 436/501, 536/25.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 10. Document ID: US 6017734 A

L7: Entry 10 of 15 File: USPT

US-PAT-NO: 6017734
DOCUMENT-IDENTIFIER: US 6017734 A

TITLE: Unique nucleotide and amino acid sequence and uses thereof

DATE-ISSUED: January 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Summers; Max D.	Bryan	TX		
Braunagel; Sharon C.	Bryan	TX		
Hong; Tao	Bryan	TX		

US-CL-CURRENT: 435/69.7; 435/320.1, 435/348, 435/365, 435/91.4, 536/23.1, 536/23.72, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 11. Document ID: US 5891430 A

L7: Entry 11 of 15 File: USPT

US-PAT-NO: 5891430
DOCUMENT-IDENTIFIER: US 5891430 A

TITLE: Diabetogene rad: a type II diabetes specific gene

DATE-ISSUED: April 6, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kahn; C. Ronald	West Newton	MA		
Reynet; Christine	Boston	MA		

US-CL-CURRENT: 424/94.6; 435/196, 530/324

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc
Image

☐ 12. Document ID: US 5846725 A

L7: Entry 12 of 15 File: USPT

US-PAT-NO: 5846725
DOCUMENT-IDENTIFIER: US 5846725 A

TITLE: Methods for identifying cisplatin resistant tumor cells

DATE-ISSUED: December 8, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yokoyama; Shiro	Machida-City, Tokyo 195			JP

US-CL-CURRENT: 435/6; 424/9.1, 424/9.34, 424/9.341, 435/25, 435/7.23, 435/7.4, 435/7.6, 436/64

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc
Image

☐ 13. Document ID: US 5837832 A

L7: Entry 13 of 15 File: USPT

US-PAT-NO: 5837832
DOCUMENT-IDENTIFIER: US 5837832 A

TITLE: Arrays of nucleic acid probes on biological chips

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chee; Mark	Palo Alto	CA		
Cronin; Maureen T.	Los Altos	CA		
Fodor; Stephen P. A.	Palo Alto	CA		
Huang; Xiaohua X.	Mt. View	CA		
Hubbell; Earl A.	Mt. View	CA		
Lipshutz; Robert J.	Palo Alto	CA		
Lobban; Peter E.	Palo Alto	CA		
Morris; MacDonald S.	San Jose	CA		
Sheldon; Edward L.	Menlo Park	CA		

US-CL-CURRENT: 536/22.1; 422/68.1, 435/6, 435/91.1, 436/501, 536/23.1, 536/24.1, 536/24.3, 536/24.31, 536/24.32, 536/24.33, 536/25.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Image | KWIC | Draw Desc

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☐ 14. Document ID: US 5646011 A

L7: Entry 14 of 15 File: USPT

US-PAT-NO: 5646011
DOCUMENT-IDENTIFIER: US 5646011 A

TITLE: Cisplatin resistance gene and uses therefor

DATE-ISSUED: July 8, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yokoyama; Shiro	Machida-City, Tokyo 195			JP

US-CL-CURRENT: 435/69.1; 435/189, 435/252.3, 435/254.1, 435/320.1, 435/325, 435/348, 536/23.2, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Image | KWIC | Draw Desc

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☐ 15. Document ID: US 5589374 A

L7: Entry 15 of 15 File: USPT

US-PAT-NO: 5589374
DOCUMENT-IDENTIFIER: US 5589374 A

TITLE: Diabetogene rad: a type II diabetes specific gene

DATE-ISSUED: December 31, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kahn; C. Ronald	West Newton	MA		
Reynet; Christine	Boston	MA		

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 536/23.2, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Image | KWIC | Draw Desc

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TUMOUR.DWPI,USPT.	21129
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Other Reference Publication (13):
M.S. Chee et al., Towards Sequencing Mitochondrial DNA Polymorphisms by Hybridization to a Custom Oligonucleotide Probe Array, American Society of Human Genetics 43rd Annual Meeting, Oct. 5-9, 1993, New Orleans, LA.

Thank You
Cynthia B. Wilder, Ph.D.
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DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02698503 Genuine Article#: LW335 No. References: 0
Title: TOWARDS SEQUENCING MITOCHONDRIAL-DNA POLYMORPHISMS BY HYBRIDIZATION TO A CUSTOM OLIGONUCLEOTIDE PROBE ARRAY
Author(s): CHEE MS; MORRIS MS; HUANG XC; DIGGLEMANN M; FODOR S
Corporate Source: AFFYMAX RES INST/SANTA CLARA//CA/95051
Journal: AMERICAN JOURNAL OF HUMAN GENETICS, 1993, V53, N3 (SEP), P985
ISSN: 0002-9297
Language: ENGLISH Document Type: MEETING ABSTRACT

983

Linkage analysis with microsatellite markers close to SCA2 locus in 6 French families: evidence for a third locus ADCA type I. ((G. Cancel, G. Stevanin, A. Dürr, H. Chneiweiss, E. Le Guern, Y. Agid and A. Brice)) * Hôpital de la Salpêtrière, Paris, France and **Collège de France, Paris, France.

Two loci responsible for autosomal dominant cerebellar ataxia type I ((ADCA) have been identified: SCA1 (spinal cerebellar ataxia 1) on chromosme 6p23-24 and more recently CSA2 on chromosome 12q23-24. Six families with ADCA type I, previously excluded from SCA1 locus were genotyped with 4 microsatellites (D12S79-D12S105-D12S84-D12S78) linked to SCA2 locus and localized in the D12S58-PLA2 interval. Bipoint and multipoint analyses generated lod scores below the threshold of -2 for the whole D12S79-D12S78 region in 5 families thereby excluding the responsability of SCA2 gene. In one family, slightly positive lod scor values did not allow to conclude. These results demonstrate that SCA2 is not the responsible gene in at least 5 of the 6 tested French families whereas whereas positive linkage to SCA1 was found in 4 others families. Exclusion of SCA1 and SCA2 loci in 5 families provide definite evidence for the existence of a third locus responsible for ADCA type I.

984

Mapping of an X-linked gene for ventral midline defects (the TAS gene). ((R.Carmi, R. Parvari, J. Weinstein.)) Soroka Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Theoretical considerations, as well as clinical observations and accumulating data in developmental biology, suggest that the human midline is a real biological entity exhibiting the properties of a developmental field. It has been suggested that the ventral midline, defined by the full spectrum of the Pentalogy of Cantrel, might present a subset of the human midline fulfilling by itself the prerequisites of a developmental field. The X-linked dominantly inherited Thoraco-Abdominal Syndrome (TAS) of ventral midline defects is suggested to be the phenotypic result of a single mutated gene operating within the putative ventral midline developmental field. It is speculated that this is a type of an early developmental gene. We have undertaken the mapping of the TAS gene in an extended family with the TAS syndrome. DNA samples were obtained from 14 affected individuals (2 males and 12 females) and 12 of their first degree relatives. A total of 45 X chromosomes were screened with highly polymorphic microsatellite (CA-repeats) probes along the X chromosome. Two-point linkage analysis between the disease locus and the X chromosomal markers excluded a location of the gene on Xp. Positive lod scores were obtained in the chromosomal region Xq22-q27. The best lod score (Zmax=5.11) was obtained for the HPRT locus (Xq26.1) at 0=0.042. Additional results indicate that the TAS gene is located between the DXS425 and HPRT loci (Xq25-q26.1).

985

Towards sequencing mitochondrial DNA polymorphisms by hybridization to a custom oligonucleotide probe array. ((M. S. Chee, M. S. Morris, X. C. Huang, M. Digglemann, and S. Fodor.)) Affymax Research Institute, 3380 Central Expressway, Santa Clara, CA 95051.

Arrays of oligonucleotide probes corresponding to overlapping regions of a consensus human mitochondrial DNA (mtDNA) sequence were synthesized on derivatized glass slides. Fluorescently labelled PCR products derived from hair root DNA were hybridized to the oligonucleotide arrays. Detection was by confocal scanning laser microscopy. Known polymorphisms between a set of cloned and sequenced mtDNA fragments were detected as differences in fluorescence.

In one design of array, a 1.3 kb region of mtDNA including the origin of replication was represented as a matrix of 269 overlapping probes varying in length from 11 to 15 nucleotides. The probe array was 1 x 1 cm in size. Sets of probes were designed to minimize cross hybridization and differences in predicted melting temperature. The synthesis site for each different probe was specifically addressed by illumination of the slide through a photolithographic mask, achieving selective deprotection (Fodor et al. (1991) Science 251, 767-773). Nucleoside phosphoramidites bearing photolabile protecting groups were then coupled to the exposed sites. Repeated cycles of deprotection and coupling were used to synthesize all the probes in parallel.

The approach is very flexible. Using custom masks, arrays can be synthesized to represent any DNA sequence of interest. The high density of probes allowsa large amount of information to be obtained from a single hybridization to a compact array.

986

DXYS156 is a polymorphic locus due to expansion of a (TAAAA) motif within a LINE repetitive element. ((H. Chen[1], W. Lowther[1,2], D. Avramopoulos[1], S. E. Antonarakis[1,2].)) [1] Center for Medical Genetics and [2] Graduate Program in Medical Genetics , The Johns Hopkins University School of Medicine , Baltimore , Maryland 21287-3914.

We report the isolation and characterization of a polymorphic pentanucleotide repeat (TAAAA)n, which was mapped to human chromosomes X and Y (locus DXYS156) by PCR amplification of DNA from a commercially available monochromosomal somatic cell hybrid panel (NIGMS panel 2). The (TAAAA)n repeat of locus DXYS156 occurs within a human LINE element at a position where the consensus sequence contains a single TAAAA motif (This single TAAAA sequence occurs at nucleotides 4284 to 4288 of the LINE sequence). In 72 unrelated CEPH individuals seven alleles were detected which ranged in size from 125 to 165 bp in 5 bp intervals. The two largest alleles (160 and 165 bp) were only observed in males which suggests that they were amplified from the Y chromosome. The other 5 alleles were present in two copies in females, and in a single copy in males, which suggests that they were amplified from the X chromosome. Locus DXYS156 was polymorphic in CEPH families with an observed heterozygosity in females of 46% (27 of 59). Linkage analysis with DNA markers on the X chromosome revealed significant lod scores for a location of DXYS156 close to markers DXS1002 (θ=0.000; Z=8.43), DXYS1X (θ =0.015; Z=17.3), DXS3 and PGK1 in the region of chromosome Xq13. It appears that the alleles from X and Y always stay specific to the corresponding chromosome, which suggests that there are no crossovers or exchange of genetic material between these regions of X and Y, unlike in the pseudoautosomal regions.

POSTER PRESENTATIONS

987

Identification of new polymorphic DNA markers closely flanking the SMA locus using homozygosity mapping.((O.Clermont, P.Burlet, L.Burglen, S.Lefebvre, F.Pascal, M.G.Lathrop, J.Weissenbach[2], A.Munnich and J.Melki.)) INSERM U.12. Hôpital des Enfants Malades, Paris.1,INSERM U.358,PARIS.2,Institut Pasteur et Genethon. Paris and Evry.

The gene for autosomal recessive forms of spinal muscular atrophy (SMA) has recently been mapped to chromosome 5q13 within a 4cM region between the blocks APM114ye7/EF5.15 and MAP-1B/JK53. Among the 138 new microsatellites assigned to chromosome 5 that were generated by one of us (J.W) , 15 were mapped to the 5q12-q14 region. A total of 50 type I, II, and III SMA families with at least two affected individuals and 29 inbred SMA families from distinct geographical region with one or two affected individuals were investigated. Conventional haplotype analysis combined with homozygosity mapping identified two new highly polymorphic microsatellites which are the closest flanking markers to the SMA locus. These data are essential for the combined genetic and physical mapping of the disease locus in order to isolate the SMA gene and demonstrate that homozygosity mapping using highly polymorphic markers may be the method of choice for high resolution mapping of recessive autosomal disorders.

988

Exclusion Mapping of Complex Disorders: A Matter of Priorities? Nancy Cox*, Carol Hempe+, Brian Suarez+. *Dept. of Medicine, U. of Chicago, Chicago, IL, +Dept. of Psychiatry, Washington University, St. Louis, MO

Conventional exclusion mapping of complex disorders with an unknown, non-Mendelian pattern of transmission is controversial. To determine whether models allowing for appreciable heterogeneity could be more useful in mapping complex disorders, we have utilized three models (a dominant, a recessive and an intermediate, maximum penetrance 0.5) allowing for a high proportion of phenocopies (70% of those affected have a non-susceptible genotype at the susceptibility locus). In two-point analyses with these models, we consider loci with a maximum lod score for any model (Zmax) > 3.4 (correction for using 3 models) to be linked to susceptibility loci; we consider loci to have no current priority for additional study if lod scores for all models are < -2.0 for theta < 0.15 and Zmax < 0.40. The remaining loci would have priority for additional study dependent on Zmax. This strategy was tested using data on nuclear families (2 parents, 4 children) ascertained through 2 affected sibs with disease susceptibility generated under a variety of complex models; each family member was "typed" at 100 fully informative marker loci, a minimum of 4 and a maximum of 10 of which were linked to susceptibility loci (theta=0 or 0.05). Heritability ranged from 50-100% and the number of families analyzed ranged from 200 to 350. General conclusions include: 1) For diseases with 100% heritability and a moderate number of contributing loci (4-6), the strategy was quite successful at identifying susceptibility loci and correctly "excluding" unlinked marker loci, at sample sizes of 200 families. 2) For diseases with 50% heritability or a large number of contributing loci (10), half of the contributing loci were falsely "excluded". While loci cannot be formally "excluded" as contributors to complex disorders, it is possible to set priorities for additional studies based on results of analyses using models allowing for heterogeneity.

L6: Entry 29 of 33 File: USPT

DOCUMENT-IDENTIFIER: US 5837832 A
TITLE: Arrays of nucleic acid probes on biological chips

Brief Summary Text (9):
The present invention provides methods for making high-density arrays of oligonucleotide probes on silica chips and for using those probe arrays to detect specific nucleic acid sequences contained in a target nucleic acid in a sample. The invention also provides arrays of oligonucleotide probes on DNA chips, in which the probes have specific sequences and locations in the array to facilitate identification of a specific target nucleic acid. In another aspect, the invention provides methods for detecting whether one or more specific sequences of a target nucleic acid in a sample varies from a previously characterized sequence or reference sequence. The methods of the invention can be used to detect variations between a target and reference sequence, including single or multiple base substitutions, and deletions and insertions of bases, as well as detecting the presence, location, and sequence of other more complex variations between a target and reference sequence in a nucleic acid.

Drawing Description Text (10):
FIG. 9 shows the human mitochondrial genome; "O.sub.H " is the H strand origin of replication, and arrows indicate the cloned unshaded sequence.

Drawing Description Text (11):
FIG. 10 shows the image observed from application of a sample of mitochondrial DNA derived nucleic acid (from the mt4 sample) on a DNA chip.

Drawing Description Text (12):
FIG. 11 is similar to FIG. 10 but shows the image observed from the mt5 sample.

Drawing Description Text (13):
FIG. 12 shows the predicted difference image between the mt4 and mt5 samples on the DNA chip based on mismatches between the two samples and the reference sequence.

Drawing Description Text (14):
FIG. 13 shows the actual difference image observed for the mt4 and mt5 samples.

Drawing Description Text (19):
FIG. 18 shows the fluorescence image produced by scanning the chip described in FIG. 17 when hybridized to a sample.

Detailed Description Text (12):
The DNA chips of the invention have a wide variety of applications. In one embodiment, the DNA chip is used to select an optimal probe from an array of probes. In this embodiment, an array of probes of variable length and sequences is synthesized and then hybridized to a target nucleic acid of known sequence. The pattern of hybridization reveals the optimal length and sequence composition of probes to detect a particular mutation or other specific sequence of nucleotides. In some circumstances, i.e., target nucleic acids with repeated sequences or with high G/C content, very long probes may be required for optimal detection. In one embodiment for detecting specific sequences in a target nucleic acid with a DNA chip, repeat sequences are detected as follows. The chip comprises probes of length sufficient to extend into the repeat region varying distances from each end. The sample, prior to

hybridization, is treated with a labeled oligonucleotide that is complementary to a repeat region but shorter than the full length of the repeat. The target nucleic is labeled with a second, distinct label. After hybridization, the chip is scanned for probes that have bound both the labeled target and the labeled oligonucleotide probe; the presence of such bound probes shows that at least two repeat sequences are present.

Detailed Description Text (50):
To demonstrate clinical application of the DNA chips of the invention, the chips were used to study and detect mutations in nucleic acids from genomic samples. Genomic samples from a individual carrying only the wild-type gene and an individual heterozygous for .DELTA.F508 were amplified by PCR using exon 10 primers containing the promoter for T7 RNA polymerase. Illustrative primers of the invention are shown below.

Detailed Description Text (58):
The present invention illustrates these advantages by providing DNA chips and analytical methods for detecting specific sequences of human mitochondrial DNA. In one preferred embodiment, the invention provides a DNA chip for analyzing sequences contained in a 1.3 kb fragment of human mitochondrial DNA from the "D-loop" region, the most polymorphic region of human mitochondrial DNA. One such chip comprises a set of 269 overlapping oligonucleotide probes of varying length in the range of 9.fwdarw.14 nucleotides with varying overlaps arranged in .about.600.times.600 micron features or synthesis sites in an array 1 cm.times.1 cm in size. The probes on the chip are shown in columnar form below. An illustrative mitochondrial DNA chip of the invention comprises the following probes (X, Y coordinates are shown, followed by the sequence; "DL3" represents the 3'-end of the probe, which is covalently attached to the chip surface.)

Detailed Description Text (59):
No probes were present in positions X, Y=0, 12 to X, Y=4, 12; X, Y=0, 13 to X, Y=4, 13; X, Y=0, 14 to X, Y=4, 14; X, Y=0, 15 to X, Y=4, 15; X, Y=0, 16 to X, Y=4, 16; The length of each of the probes on the chip was variable to minimize differences in melting temperature and potential for cross-hybridization. Each position in the sequence is represented by at least one probe and most positions are represented by 2 or more probes. As noted above, the amount of overlap between the oligonucleotides varies from probe to probe. FIG. 9 shows the human mitochondrial genome; "O.sub.H " is the H strand origin of replication, and arrows indicate the cloned unshaded sequence.

Detailed Description Text (61):
The sample nucleic acid was hybridized to the chip in a solution composed of 6.times.SSPE, 0.1% Triton-X 100 for 60 minutes at 15.degree. C. The chip was then scanned by confocal scanning fluorescence microscopy. The individual features on the chip were 588.times.588 microns, but the lower left 5.times.5 square features in the array did not contain probes. To quantitate the data, pixel counts were measured within each synthesis site. Pixels represent 50.times.50 microns. The fluorescence intensity for each feature was scaled to a mean determined from 27 bright features. After scanning, the chip was stripped and rehybridized; all six samples were hybridized to the same chip. FIG. 10 shows the image observed from the mt4 sample on the DNA chip. FIG. 11 shows the image observed from the mt5 sample on the DNA chip. FIG. 12 shows the predicted difference image between the mt4 and mt5 samples on the DNA chip based on mismatches between the two samples and the reference sequence (see Anderson et al., 1981, Nature 290: 457-465, incorporated herein by reference). FIG. 13 shows the actual difference image observed.

Detailed Description Text (66):
FIG. 17 provides a 5' to 3' sequence listing of one target corresponding to the probes on the chip. X is a control probe. Positions that differ in the target (i.e., are mismatched with the probe at the designated site) are in bold. FIG. 18 shows the fluorescence image produced by scanning the chip when hybridized to this sample. About 95% of the sequence could be read correctly from only one strand of the original duplex target nucleic acid. Although some probes did not provide excellent discrimination and some probes did not appear to hybridize to the target efficiently, excellent results were achieved. The target sequence differed from the probe set at six positions: 4 transitions and 2 insertions. All 4 transitions were detected, and

specific probes could readily be incorporated into the array to detect insertions or deletions. FIG. 19 illustrates the detection of 4 transitions in the target sequence relative to the wild-type probes on the chip.

Detailed Description Text (67):
These results illustrate that longer sequences can be read using the DNA chips and methods of the invention, as compared to conventional sequencing methods, where reading length is limited by the resolution of gel electrophoresis. Similar results were observed when genomic DNA samples were prepared from human hair roots. Hybridization and signal detection require less than an hour and can be readily shortened by appropriate choice of buffers, temperatures, probes, and reagents. In principle, longer sequence reads can be obtained than by conventional sequencing, where reading length is limited by the resolution of gel electrophoresis.

Detailed Description Text (69):
P53 is a tumor suppressor gene that has been found to be mutated in most forms of cancer (see Levine et al, 1991, Nature 351: 453-456, and Hollstein et al., 1991, Science 253: 49-53, each of which is incorporated herein by reference). In addition, there is a hereditary syndrome, Li-Fraumeni, in which individuals inherit mutant alleles of p53 and tend to have cancer at relatively young ages (Frebourg et al., 1992, PNAS 89: 6413-6417, incorporated herein by reference). During the development of a cancer, p53 is inactivated. The course of p53 inactivation generally involves a mutation in one copy of p53 and is often followed by deletion of the other copy. After p53 is inactivated, chromosomal abnormalities begin to appear in tumors. In the best understood form of cancer, colorectal cancer, well over 50%, perhaps 80%, of all patients with tumors have p53 mutations. In addition, p53 mutations have been found in a high proportion of lung, breast, and other tumors (Rodrigues et al., 1990, PNAS 87: 7555-7559, incorporated herein by reference). According to data presented by David Sidransky (1992 San Diego Conference), over 400 mutations in p53 are known.

Detailed Description Text (70):
The p53 gene spans 20 kbp in humans and has 11 exons, 10 of which are protein coding (see Tominaga et al., 1992, Critical Reviews in Oncogenesis 3: 257-282, incorporated herein by reference). The gene produces a 53 kilodalton phosphoprotein that regulates DNA replication. The protein acts to halt replication at the G1/S boundary in the cell cycle and is believed to act as a "molecular policeman," shutting down replication when the DNA is damaged or blocking the reproduction of DNA viruses (see Lane, 1992, Nature 358: 15-16, incorporated herein by reference). There is substantial interest in the cancer research community in analyzing p53 mutations. The NCI is currently funding contracts to characterize the p53 mutation spectra caused by various carcinogens. In addition, there are research projects which involve sequencing p53 from spontaneously arising tumors. A major resource in these studies is the huge supply of biopsy material stored in paraffin blocks. Also, there are projects which are aimed at analyzing the relationship between the particular mutation in p53 and the functioning of the resulting protein. Furthermore, there are projects looking at the germline inheritance of p53 mutations and the development of cancer. The present invention provides useful DNA chips and methods for such studies.

Detailed Description Text (71):
In addition, the present invention also provides a diagnostic test kit and method and p53 probes immobilized on a DNA chip in an organized array. Currently available diagnostic tests for cancer typically have a sensitivity of about 50%. The present invention provides significant advantages over such tests, and in one embodiment provides a method for detecting cancer-causing mutations in p53 that involves the steps of (1) obtaining a biopsy, which is optionally fractionated by cryostat sectioning to enrich tumor cells to about 80% of the total cell population. The DNA or RNA is then extracted, amplified, and analyzed with a DNA chip for the presence of p53 mutations correlated with malignancy.

Detailed Description Text (76):
When a target with a different one base substitution is hybridized the complementary set of probes has the highest signal (see pictures 2, 3, and 4 in FIG. 22 and graphs 2, 3, and 4 in FIG. 23). In each case, the probe set with no mismatches with the target has the highest signals. Within a 12-mer probe set, the signal was highest at position 6 or 7. The graphs show that the signal difference between 12-mer probes at

the same X ordinate tended to be greatest at positions 5 and 8 when the target and the complementary probes formed 10 base pairs and 11 base pairs, respectively. Because tumors often have both WT and mutant p53 genes, mixed target populations were also hybridized to the chip, as shown in FIG. 24. When the hybridization solution consisted of a 1:1 mixture of WT 12-mer and a 12-mer with a substitution in position 7 of the target, the sets of probes that were perfectly matched to both targets showed higher signals than the other probe sets.

Detailed Description Text (79):
For sequencing, the p53 DNA can be cloned from the sample or directly amplified from genomic DNA by PCR. If genomic PCR is used, then the DNA can be diluted prior to amplification so that a single copy of the gene is amplified. For diagnostic purposes, the genomic DNA can be isolated from a tumor biopsy in which the tumor cells may be the majority population. As noted above, the proportion of tumor cells in a sample can be enriched by cryostat sectioning. DNA can also be isolated and amplified from tumor samples stored in paraffin blocks.

Detailed Description Text (80):
The p53 DNA in the sample can be amplified by PCR (although other amplification methods can be used) using 3-4 primer pairs generating amplicons of <3 kbp each. Illustrative primers of the invention for amplifying exon 5 of the p53 gene are shown below (B is biotin; F is fluorescein).

Detailed Description Text (90):
The invention is illustrated below with examples of DNA chips comprising very large arrays of DNA probes to "resequence" p53 target nucleic acid in a sample. To analyze DNA from exon 5 of the p53 tumor suppressor gene, a set of overlapping 17-mer probes was synthesized on a chip. The probes for the WT allele were synthesized so as to tile across the entire exon with single base overlaps between probes. For each WT probe, a sets of 4 additional probes, one for each possible base substitution at position 7, were synthesized and placed in a column relative to the WT probe. Exon 5 DNA was amplified by PCR with primers flanking the exon. One of the primers was labeled with fluorescein; the other primer was labeled with biotin. After amplification, the biotinylated strand was removed by binding to streptavidin beads. The fluoresceinated strand was used in hybridization.

Detailed Description Text (92):
As the diagram indicates, the miscalled bases are from the low intensity areas of the image, which may be due to secondary structure in the target or probes preventing intermolecular hybridization. To diminish the effects due to secondary structure, one can employ shorter targets (i.e., by target fragmentation) or use more stringent hybridization conditions. In addition, the use of a set of probes synthesized by tiling across the other strand of a duplex target can also provide sequence information buried in secondary structure in the other strand. It should be appreciated, however, that the pattern of low intensity areas that forms as a result of secondary structure in the target itself provides a means to identify that a specific target sequence is present in a sample. Other factors that may contribute to lower signal intensities include differences in probe densities and hybridization stabilities.

Detailed Description Text (93):
These results demonstrate the advantages provided by the DNA chips of the invention to genetic analysis. As another example, heterozygous mutations are currently sequenced by an arduous process involving cloning and repurification of DNA. The cloning step is required, because the gel sequencing systems are poor at resolving even a 1:1 mixture of DNA. First, the target DNA is amplified by PCR with primers allowing easy ligation into a vector, which is taken up by transformation of E. coli which in turn must be cultured, typically on plates overnight. After growth of the bacteria, DNA is purified in a procedure that typically takes about 2 hours; then, the sequencing reactions are performed, which takes at least another hour, and the samples are run on the gel for several hours, the duration depending on the length of the fragment to be sequenced. By contrast, the present invention provides direct analysis of the PCR amplified material after brief transcription and fragmentation steps, saving days of time and labor.

Detailed Description Text (94):
An interesting clinical application for the characterization of heterozygous mutations with DNA chips is as follows. Individuals with germline cancer mutations have a very high risk for secondary tumors after treatment by irradiation. About 10% of all cancer patients may have germline mutations for p53 or other tumor suppressor genes. Thus, before deciding on a treatment modality, a physician could use the method and DNA chips of the invention to test for a germline suppressor gene mutation.

Detailed Description Text (98):
The present invention provides DNA chips for detecting the multiple mutations in the HIV RT gene associated with resistance to different therapeutics. These DNA chips will enable physicians to monitor mutations over time and to change therapeutics if resistance develops. The DNA chip will provide redundant confirmation of conserved HIV RT and other gene sequences, and the probes on the chip will tile through, with overlap, in important mutational hot spot regions. The chip will optionally have probes that span the entire coding region of the RT and optionally the genes for other HIV proteins, such as coat proteins. HIV target nucleic acid can be isolated from blood samples (peripheral blood lymphocytes or PBMC) and amplified by PCR, primers for which are shown in the table below.

Detailed Description Text (99):
The HIV RT gene chips of the invention, as well as the CF, mtDNA, and p53 DNA chips of the invention, illustrate the diverse application of the methods and probe arrays of the invention. The examples that follow describe methods for preparing nucleic acid targets from samples for application to the DNA chips of the invention and provide additional details of the methods of the invention.

Detailed Description Text (129):
PCR amplification reactions are typically conducted in a mixture composed of per reaction: 1 .mu.l genomic DNA; 10 .mu.l each primer (10 pmol/.mu.l stocks); 10 .mu.l 10.times.PCR buffer (100 mM Tris.Cl pH8.5, 500 mM KCl, 15 mM MgCl.sub.2); 10 .mu.l 2 mM dNTPs (made from 100 mM dNTP stocks); 2.5 U Taq polymerase (Perkin Elmer AmpliTaq.TM., 5 U/.mu.l); and H.sub.2 O to 100 .mu.l. The cycling conditions are usually 40 cycles (94.degree. C. 45 sec, 55.degree. C. 30 sec, 72.degree. C. 60 sec) but may need to be varied considerably from sample type to sample type. These conditions are for 0.2 mL thin wall tubes in a Perkin Elmer 9600 thermocycler. See Perkin Elmer 1992/93 catalogue for 9600 cycle time information. Target, primer length and sequence composition, among other factors, may also affect parameters.

Detailed Description Text (131):
For simple amplifications of short fragments from genomic DNA it is, in general, unnecessary to optimize Mg.sup.2+ concentrations. A good procedure is the following: make a master mix minus enzyme; dispense the genomic DNA samples to individual tubes or reaction wells; add enzyme to the master mix; and mix and dispense the master solution to each well, using a new filter tip each time.

Detailed Description Text (133):
Removal of unincorporated nucleotides and primers from PCR samples can be accomplished using the Promega Magic PCR Preps DNA purification kit. One can purify the whole sample, following the instructions supplied with the kit (proceed from section IIIB, `Sample preparation for direct purification from PCR reactions`). After elution of the PCR product in 50 .mu.l of TE or H.sub.2 O, one centrifuges the eluate for 20 sec at 12,000 rpm in a microfuge and carefully transfers 45 .mu.l to a new microfuge tube, avoiding any visible pellet. Resin is sometimes carried over during the elution step. This transfer prevents accidental contamination of the linear amplification reaction with `Magic PCR` resin. Other methods, e.g. size exclusion chromatography, may also be used.

Detailed Description Text (154):
For obtaining genomic DNA from human hair, one can extract as few as 5 hairs, including hair roots. On a clean and sterile surface, one places the hair on a piece of parafilm, and after wiping a new razor blade with EtOH cutting off the roots, the roots are transferred to a 1.5 mL microfuge tube using a pair of Millipore forceps cleaned with EtOH. Add 500 .mu.l (10 mM Tris.Cl pH8.0, 10 mM EDTA, 100 mM NaCl, 2% (w/v) SDS, 40 mM DTT, filter sterilized) to the sample. Add 1.25 .mu.l 20 mg/ml

proteinase K (Boehringer) Incubate at 55.degree. C. for 2 hours, vortexing once or twice. Perform 2.times.0.5 mL 1:1 phenol:CHCl.sub.3 extractions. After each extraction, centrifuge 12,000 rpm 5 min in a microfuge and recover 0.4 mL supernatant. Add 35 .mu.l NaAc pH5.2 plus 1 mL EtOH. Place sample on ice 45 min; then centrifuge 12,000 rpm 30 min, rinse, air dry 30 min, and resuspend in 100 .mu.l TE.

Other Reference Publication (13):
M.S. Chee et al., Towards Sequencing Mitochondrial DNA Polymorphisms by Hybridization to a Custom Oligonucleotide Probe Array, American Society of Human Genetics 43rd Annual Meeting, Oct. 5-9, 1993, New Orleans, LA.